The Chemical Dynamics of Serum Reactions.

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A multitude of phenomena of widely varying character is seen in experimenting with blood sera: Agglutination, bacteriolysis, hæmolysis, precipitin, opsonin, and stimulin reactions, and others occur.

In the serum two elements are distinctly recognisable: (1) A so-called *amboceptor*, which is characterised by its being able to withstand comparatively high temperatures, and by the fact that it can act alone.

(2) A so-called *complement*, which is put out of action by comparatively low temperatures, and which cannot act unless the amboceptor has previously acted.

It is the object of this paper to show that the above reactions are subject to the law of mass action, and that they can be expressed by the whole or part of the equation

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz} - z\right) - \left(\frac{y}{c} - z\right)^2, \tag{1}$$

according as both elements are present, or only amboceptor is present.

y = per cent. of amboceptor,
x = „ complement,
c = a constant of dilution,
z = a complex quantity, denoting effect, as observed in any of the above reactions,
t = time.

This formula merely states that amboceptoral action is bimolecular, and that complement action is monomolecular, under the influence of a catalytic effect of the amboceptor.

Writing the equation thus:

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{ca} - z\right) - \left(\frac{y}{c} - z\right)^n \tag{2}$$

is the mathematical form of stating the characters of the two components (as given above) with the hypothesis that amboceptoral action is directly

opposed to complement *plus* catalyst action—not a direct opposition in the fluid, but an opposition in their action on the cell.

$\frac{y}{c} - z$ = concentration of free amboceptor,

$\frac{x}{ca} - z$ = concentration of free complement,

$\frac{y}{c}$ = concentration of amboceptor, both free and combined (*i.e.* the concentration in which it acts catalytically).

The fundamental equation (1) was obtained from a study of the value of z got by mixing varying proportions of y and x.

The details of experiment are as follows:—The materials used were human serum for the serum components, and the cells were washed horse cells. Three fluids were made up:—

	Unheated serum.	Heated serum.	*i.e.* y.	x.
	parts.	parts.		
Fluid *a*	4	0	4	4
„ *b*	2	2	4	2
„ *c*	1	3	4	1

A series of dilutions of each of these fluids was put up as follows:—

	Fluid *a*, *b*, or *c*.	0·85-per-cent. NaCl solution.	Washed cells.	*I.e.* per cent. amboceptor.
	c.c.	c.c.	c.c.	
1	2·45	0·0	0·05	98·0
2	2·05	0·4	0·05	82·0
3	1·8	0·65	0·05	72·0
4	1·55	0·9	0·05	62·0
5	1·3	1·15	0·05	52·0
6	1·05	1·4	0·05	42·0
7	0·8	1·65	0·05	32·0
8	0·55	1·9	0·05	22·0
9	0·5	1·95	0·05	20·0
10	0·44	2·01	0·05	17·6
11	0·375	2·065	0·05	15·0
12	0·31	2·14	0·05	12·5
13	0·25	2·2	0·05	10·0
14	0·187	2·263	0·05	7·5
15	0·125	2·325	0·05	5·0
16	0·0625	2·3875	0·05	2·5
17	0·0	2·45	0·05	0·0

I used this arrangement of combinations of heated and unheated sera because by it the area in which the phenomenon of diversion occurs, whose characteristics I had set out particularly to investigate, can be explored and

disclosed to view. The tubes were incubated at 37° C. for one hour, and were placed on ice overnight.

The observed results are given in Table I for the lowest complete lysis, in Table II for the lowest partial lysis. The first two columns give observed values, and the third column contains the corresponding values calculated by means of the fundamental equation, which will be referred to later.

Table I.—For Complete Lysis.

y and x varying; z constant; $dz/dt = 0$ (*i.e.* when the reaction has ceased). $c = 11{\cdot}37$, $z = 1{\cdot}256$ (the method by which these constants were obtained will be explained at the end of this section after the mathematical discussion).

y.	x observed.		x calculated.
per cent.	per cent.	per cent.	per cent.
5·0			39·54
10·0			20·23
14·281*	>14·0		17·936
20·0		20·0	19·54
30·0		<30·0	28·27
40·0		<40·0	38·72
50·0		<50·0	49·98
52·5		<52·5	52·88
55·0	about 55·0		55·85
62·5	>62·5		64·66

* "Point of diversion."

Table II.—For Lowest Partial Lysis.

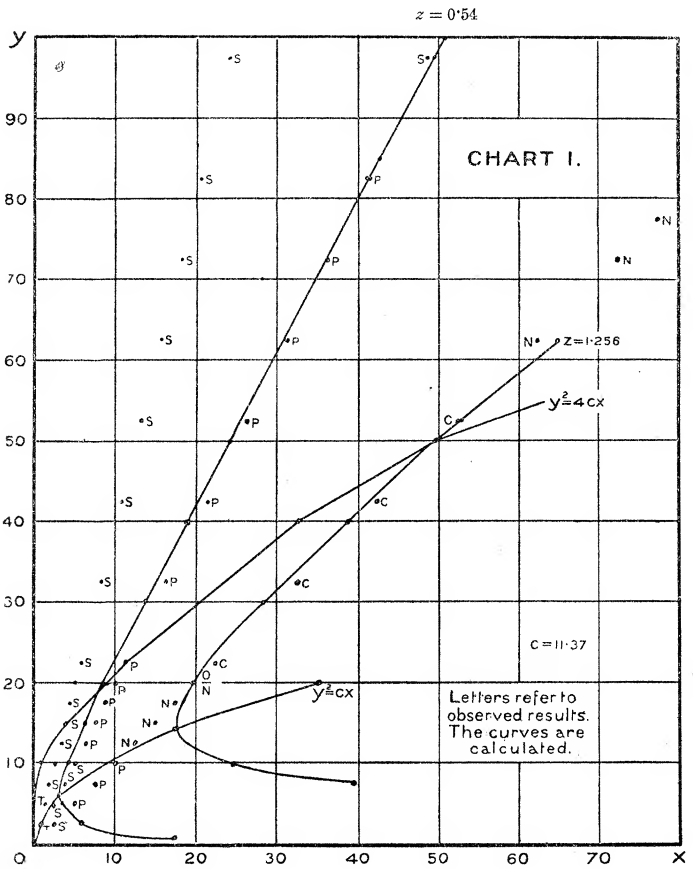
Conditions as in Table I. $c = 11{\cdot}37$, $z = 0{\cdot}54$.

y.	x observed.		x calculated.
per cent.	per cent.	per cent.	per cent.
1·0			17·59
2·5			6·06
5·0	>2·5	<5·0	3·45
6·1398*			3·31
10·0	>5·0		4·22
15·0	>3·75	< 7·5	6·14
20·0		<10·0	8·50
30·0	>7·5	<15·0	13·56
40·0		<20·0	18·79
50·0		<25·0	24·09
82·5	about 41·25		41·48
85·0	about 42·25		42·72
97·5	>48·75		49·54
100·0			50·89

* "Point of diversion."

The results of this experiment are shown in Chart 1, where points—

Marked C indicate complete lysis.
„ N „ nearly complete lysis.
„ P „ partial lysis.
„ S „ slight „
„ T „ a trace of lysis.



It is particularly to be noticed that the curves are thrown back (towards the right) in their upper parts. This exhibits the special phenomenon (already referred to) called "complement diversion." As the curves appeared to form a regular series, I considered that the principle lying at the root of serum reactions was to be found in this phenomenon.

Assuming the curves to be hyperbolic from their appearance when z was constant, I looked for the asymptotes. One asymptote was obviously $y = 0$; another suggests itself as of the form $x/z - y = 0$; for, letting z

take smaller and smaller values, this asymptote approached closer and closer to $x-0=0$. At the same time, it was clear that, in general, these inclined asymptotes cut the axis of y at some point other than the origin; and, on the other hand, this point of intersection reached the origin when z became zero that is, there was no lysis in the absence of both amboceptor and complement. I chose, then, as the final equation for this asymptote,

$$\frac{x}{z}-y+cz=0.$$

Hence the equation of any of these curves, in which z was constant, became

$$y\left(\frac{x}{z}-y+cz\right)=d, \tag{3}$$

where d was in general some function of z, to be determined.

The experimental curves showed that the lytic value for any one of these curves could not be attained if the complement fell below a minimal amount, depending on the constant value of the lysis for the particular curve in question. Seeing that the phenomenon of "diversion" begins here, the corresponding point on any hyperbola may appropriately be called its "point of diversion." At a point of diversion the tangent to the hyperbola is parallel to the axis of y; hence to find these points we have only to find x and y from equation (3), and from the equation got from putting $dx/dy=0$ derived from it when z, and therefore d, are constant. This latter equation is

$$\frac{x}{z}-2y+cz=0. \tag{4}$$

Whence, by means of (3), we get at once

$$y=\sqrt{d}, \quad \text{and} \quad x=2z\sqrt{d}-cz^2 \tag{5}$$

the co-ordinates of the "point of diversion" in terms of z.

The co-ordinates of the "point of diversion" of any of these hyperbolas satisfy equation (4) for $z=$ constant. The envelope of this line, for variations of z, is

$$y^2=cx. \tag{6}$$

If we assume that all "points of diversion" lie on this parabolic cylinder—a view which, at least, seems favoured by the appearance of the curves—we are able to determine d as a function of z. For, writing equation (4) in the form

$$x-2yz+cz^2=0,$$

by equation (6) it becomes

$$y^2-2ycz+c^2z^2=0, \quad \text{or} \quad y=cz, \tag{7}$$

whence

$$d=c^2z^2.$$

and equation (3) takes the form

$$y\left(\frac{x}{z}-y+cz\right)=c^2z^2,$$

or, finally,

$$\frac{y}{c}\left(\frac{x}{cz}-z\right)-\left(\frac{y}{c}-z\right)^2=0. \qquad (8)$$

At a *point of diversion*, where $dz/dy = 0$, we have the equations

$$y^2 = cx,\ y = cz, \quad \text{whence} \quad x = cz^2,\ x = yz. \qquad (6, 7, 9, 10)$$

Further, when x is constant, $dz/dy = 0$ leads in a similar manner to equation (4) and the associated equations (6, 7, 9, 10). These last two results can easily be seen from the geometry alone of the figure.

Equation (8) is the equilibrium condition of the theoretical equation (2), with the substitution of z in place of a and 2 in place of n.

Equation (1)

$$\frac{dz}{dt}=\frac{y}{c}\left(\frac{x}{cz}-z\right)-\left(\frac{y}{c}-z\right)^2$$

has now been arrived at. To prove that it is correct, it must agree with experimental results for all values of y, x, z, and t. I shall show from certain of these that it does so.

If experiments be well planned, the constants c and z can be obtained with ease. The simplest mode of procedure is to erect an experiment on a line $x =$ constant, choosing a low value of x. The concentration of y, which gives the greatest degree of lysis, fulfils the requirements of equations (6), (7), (9), (10), for $y^2 = cx$ is the family curve of the points of diversion, and the tangent at the point of diversion is parallel to the y axis.

If a small series of this sort be put up, the tube which shows the first flash of lysis may be taken as an approximation, and a further series of tubes with concentrations differing only slightly from this may be put up forthwith. When the concentration of y which causes maximum lysis in the series has been determined, the equation $y^2 = cx$ evaluates c. The maximum degree of lysis having been evaluated colorimetrically, the equation $x = yz$ gives the value of z for that degree of lysis.

In the experiment which I have described, another method had to be adopted. As I have shown, the asymptote for a constant value of z is $x/z-y+cz = 0$, and its slope $dx/dy = z$.

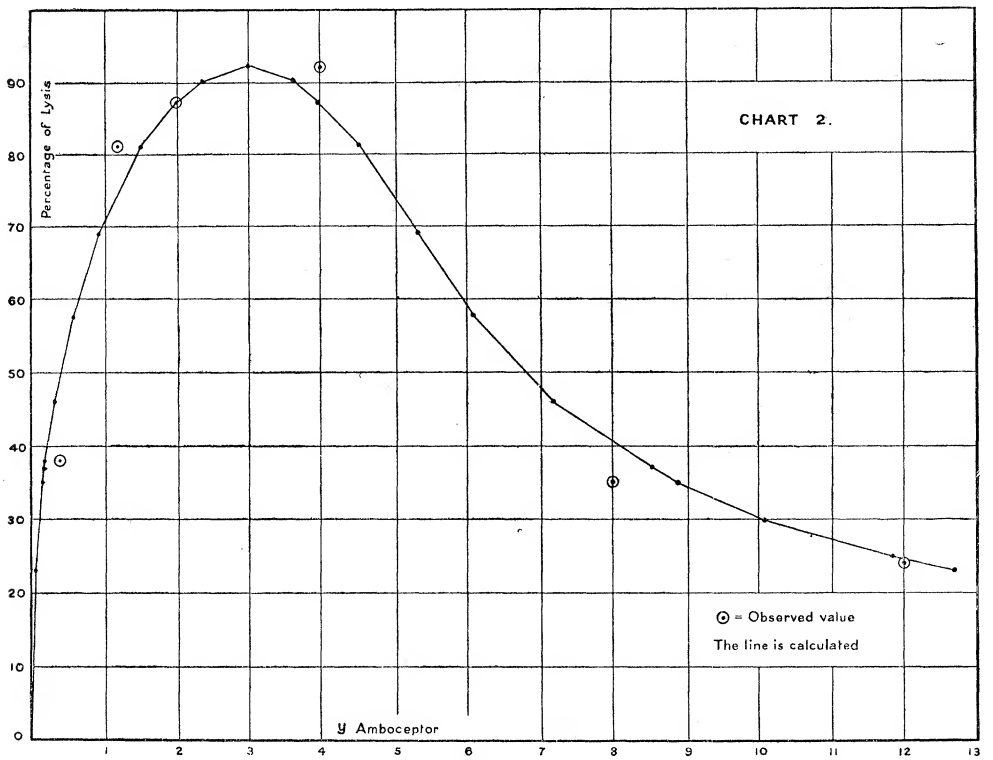
In the series (Chart 1) erected on the line $x = y$, complete lysis values are succeeded by incomplete in the upper half of the line. Thus, z for complete lysis is greater than 1. I chose its value at 1·25.

In the series erected on the line $x = 0{\cdot}5y$, partial lysis values are succeeded in the upper part of the line by a value "slight—partial." Thus, z for partial lysis is slightly greater than 0·5. I chose its value as 0·55.

The point of diversion for the curve of complete lysis appeared to lie near the point ($y = 15, x = 19$), from equation (6) $y^2 = cx$; thus, $c = 11{\cdot}8$. These approximations were subsequently modified to $c = 11{\cdot}37$, and

z for complete lysis $= 1{\cdot}256$,
z for lowest partial lysis $= 0{\cdot}54$.

II. y and z varying; x constant. $dz/dt = 0$. *Vide* Chart 2.—The following experiment is taken from Arrhenius ('Immuno Chemistry,'



English Edition, p. 225), for his value $b = 6$, *i.e.*, $x = 0{\cdot}24$ per cent. I have expressed his units in percentages, *e.g.*, $y = 2$ means $a = 50$:—

Percentage lysis.	y used.	y calculated.
37	0·4	0·169
81	1·2	1·52
87	2·0	2·01
92	4·0	3·0
35	8·0	8·9
24	12·0	12·14

$c = 37{\cdot}5$, $z = 0{\cdot}08$ for 92 per cent. lysis.

The constants were obtained as follows:—

The summit of the observed curve (*vide* Chart 2) is shown as lying between 3 and 4. I chose $y = 3$ as the summit, and that the degree of lysis at this point was 92 per cent. The value of x is 0·24 throughout the whole curve. The summit conditions are given by equations (6) and (7).

By equation (6) $y^2 = cx$

we have $c = 9/0{\cdot}24,$

or $c = 37{\cdot}5.$

By equation (7) $y = cz,$

$z = 3/37{\cdot}5.$

Therefore $z = 0{\cdot}08$ for 92 per cent. lysis.

A comparison of observed and calculated values in Chart 2 shows more clearly than the figures do that all the singularities of the curve are fulfilled.

III. x and z varying; amboceptor (y) constant. $dz/dt = 0$. Chart 3.—To find the constants in the (x, z) plane is much more difficult. A point of flexion may be found within the range of experiment. When little amboceptor is present it lies near the y axis. When much more amboceptor is present it is not seen; the curve is nearly flat; the point, as a matter of fact, lies beyond the line of complete lysis.

The curve thus occurs in widely different forms. For low values of y it is almost entirely convex upwards. For high values of y it is concave upwards, in reality nearly flat, and for intermediate values it assumes an $\int$ form. Some of these forms are shown in Chart 3.

The point of flexion can be found mathematically as follows. Re-arrange equation (8)

$$x + cz^2 = yz + c^2z^3y^{-1};$$

thus
$$\frac{dz}{dx} = \frac{1}{y + 3c^2z^2y^{-1} - 2cz},$$

and
$$\frac{d^2z}{dx^2} = \frac{2c - 6c^2zy^{-1}}{(y + 3c^2z^2y^{-1} - 2cz)^2}\frac{dz}{dx}.$$

When $\frac{d^2z}{dx^2} = 0$ (the point sought for),

either $\frac{dz}{dx} = 0$, which is obviously not the case,

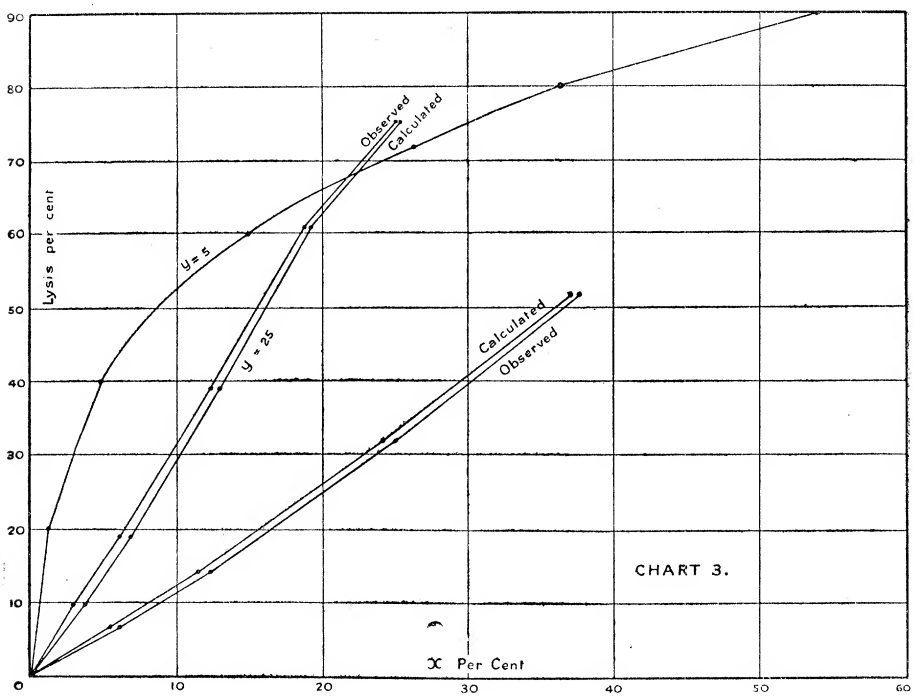
or
$$6c^2zy^{-1} = 2c, \quad i.e. \quad y = 3cz. \tag{11}$$

Substituting this value of y in (8), we find

$$x = \tfrac{7}{3}cz^2. \tag{12}$$

Consequently,

$$\begin{cases} y^2 = 3{\cdot}8571cx, & (13) \\ x = \frac{7}{9}yz. & (14) \end{cases}$$



The following experiment shows these facts very clearly. Normal human serum was made up in the following proportions:—

	Unheated serum.	Heated serum.	*I.e.* y.	x.
	parts.	parts.		
A	4	0	4	4
B	3	1	4	3
C	2	2	4	2
D	1	3	4	1
E	1	7	8	1

Tubes were put up in two series.

	c.c.
Series 1.—Serum mixture (A, B, C, D, or E)	1·00
0·85-per-cent. NaCl solution	0·95
Washed horse cells	0·05
Total	2·00

i.e. in this series amboceptor = 50 per cent.

	c.c.
Series 2.—Serum mixture (A, B, C, D, or E)	0·50
0·85-per-cent. NaCl solution.....	1·45
Washed horse cells	0·05
Total.....	2·00

i.e. in this series amboceptor = 25 per cent.

The tubes were incubated at 37 C. for *two hours*, and kept on ice overnight. They were then estimated for lysis by colorimetry.

The results are as follows (*vide* Chart 3):—

		Observed lysis.	z.	x used.	x calculated.
Series 1. $y = 50$ ...	B	52·0	0·86	37·5	36·87
	C	32·0	0·5$\dot{3}$	25·0	24·12
	D	14·6	0·24$\dot{3}$	12·5	11·603
	E	6·8	0·11$\dot{3}$	6·25	5·54
Series 2. $y = 25$ ...	A	80·4	1·34	25·0	25·16
	B	61·0	1·01$\dot{6}$	18·75	19·284
	C	39·2	0·65$\dot{3}$	12·5	13·17
	D	19·0	0·31$\dot{6}$	6·25	7·02
	E	9·8	0·16$\dot{3}$	3·125	3·83

Constants $c = 10$, $z = 0{\cdot}8\dot{3}$ for 50 per cent. lysis.

The constants were obtained as follows:—

The observed results having been charted it was observed that in the curve for ($y = 25$) there was a point of inflexion whose co-ordinates were approximately (Lysis = 50 per cent.) ($x = 15{\cdot}5$).

By equation (11) $y = 3cz$, thus $cz = 8{\cdot}\dot{3}$.

By equation (12) $x = \frac{7}{3}cz^2$, thus $z = 0{\cdot}797$.

Substituting this value in (11),

$$c = 8{\cdot}\dot{3}/0{\cdot}797 = 10{\cdot}45.$$

These approximations were subsequently modified to $c = 10$ and $z = 0{\cdot}8\dot{3}$ for 50 per cent. lysis.

Chart 3 shows more remarkably than the tabulated results do, how complete is the accordance between the theoretical and the observed forms of curve. (In the lowest curve in Chart 3, $y = 50$.)

The $\int$ form, with its point of flexion, is shown for the value $y = 25$, and the preliminary concavity for the higher value $y = 50$. An experimental point of note is that for observed results to agree with the equation of equilibrium, it is absolutely necessary that the reaction should be allowed to

continue for a sufficient period of time. One hour at 37° C. is insufficient; under such conditions the bulges in the $\int$ form are much more developed, but the curve is not calculable by equation (8), as the system is not in equilibrium. I have found that incubation for two hours at 37° C., followed by 12 hours in the ice box, is sufficient for practical purposes.

The variables y, x, and z, have now been fully observed; and the surface obtained by the equation of equilibrium agrees in every detail and in all its singular properties with the form obtained from experiment.

IV. y and t varying. Complement absent ($x = 0$); effect z constant. This leads us into a different domain of serum reactions, viz., agglutination. Equation (1) loses its fraction dependent on concentration of complement, and becomes

$$\frac{dz}{dt} = -\left(\frac{y}{c} - z\right)^2. \tag{15}$$

On integration $$-\frac{yt}{c} = \frac{z}{\frac{y}{c} - z} \quad \text{or} \quad yt = \frac{c^2 z}{cz - y}. \tag{16}$$

The appended table is taken from Arrhenius,* and I have added as a fourth column results calculated from the right hand of equation (16)—

y	t (minutes).	yt	$c^2z/(cz-y)$.
0·008	140	(1·12)	1·11
0·006	150	0·90	0·95
0·0055	165	0·91	0·912
0·005	180	0·90	0·888
0·004	210	0·84	0·83
0·0035	240	0·84	0·80
0·003	300	0·90	0·784
0·0022	420	0·92	0·75
0·002	480	0·96	0·74

$c = 0·66$, $z = 0·03$.

These constants were obtained by solving simultaneous equations obtained by introducing observed values of y and t in equation (16).

Arrhenius considers that there is an experimental error in the first value of yt, due to the fact that the liquids were not immediately brought to a constant temperature, and considers yt to be constant (mean for last eight values = 0·90). I would suggest that the discrepancy lies in the later figures. Agglutination is measured by the eye. When the reaction is fast the flocculi are larger and are more easily observed. When the reaction is slow agglutination occurs in smaller, more discrete particles, and observed times

* *Loc. cit.*, p. 104.

would tend to be too long. (I have presumed that, in this experiment, the number of bacteria was sufficient to take up all the amboceptor. I shall discuss this in Section VIII.)

V. *The Relation between y and x which gives a Maximum Velocity.*—

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz} - z\right) - \left(\frac{y}{c} - z\right)^2, \qquad \frac{d}{dy}\left(\frac{dz}{dt}\right) = \frac{x}{c^2 z} + \frac{z}{c} - \frac{2y}{c^2},$$

when

$$\frac{d}{dy}\left(\frac{dz}{dt}\right) = 0$$

(*i.e.*, when increase of velocity per increment in y is zero), then

$$2y = x/z + cz,$$

and this equation is satisfied when

$$y = cz, \quad x = cz^2, \quad y^2 = cx, \quad x = zy,$$

i.e. at "points of diversion."

This conclusion is made use of in the method of finding the approximate value of c, as described at the end of Section I.

VI.—*When the Reaction is performed in Two Stages* (Supersensitisation phenomena).—Let y be $> cz$. The first part of the experiment follows the equation

$$\frac{dz}{dt} = -\left(\frac{y}{c} - z\right)^2.$$

When saturation has been accomplished,

$$\frac{dz}{dt} = 0, \qquad t = \infty, \qquad \frac{y}{c} = z.$$

If z for complete lysis was z_0 at the commencement of the reaction, it is after the first part of the experiment equal to $y/c \,.\, z_0$ (call this z_1).

If complement be now added, the reaction follows the equation

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz_1} - z_1\right).$$

(The value y/c is retained as all the amboceptor has become attached.)

When equilibrium has been reached, $x = cz_1^2$.

Had the experiment been performed in the usual manner in the presence of both components, equilibrium would have been reached when

$$\left(\frac{y}{c} - z_0\right)^2 = \frac{y}{c}\left(\frac{x}{cz_0} - z_0\right).$$

That is to say the "point of diversion" would have been at

$$y = cz_0, \qquad x = cz_0^2.$$

Thus the value of x at the point of diversion in the divided experiment would be

$$x_1 = c\left(\frac{y}{c}z_0\right)^2;$$

in the simultaneous experiment

$$x_0 = cz_0^2.$$

The state of affairs is shown simply in the accompanying diagram :—

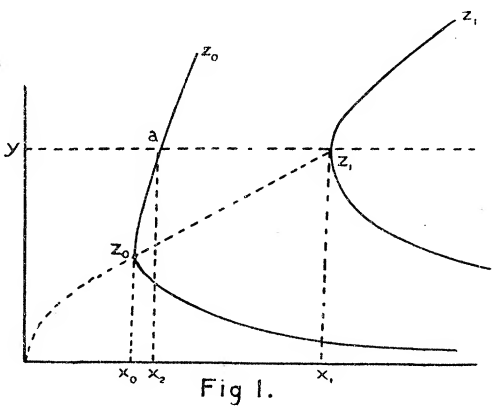


Fig 1.

Let us suppose that the serum employed contained y of amboceptor and x_2 of complement. In an ordinary simultaneous experiment, complete lysis would occur at the point a, the curve for complete lysis being the line az_0.

If, however, the experiment is performed in two stages, the initial action of amboceptor y is to shift the line of complete lysis from z_0 to z_1. Consequently, the line z_0 will now be a line of partial lysis, the available quantity of complement x_2 being insufficient to lyse the supersensitised cells. If x_2 be increased to x_1, complete lysis will be attained. An advanced degree of this phenomenon is shown in the following experiment :—*

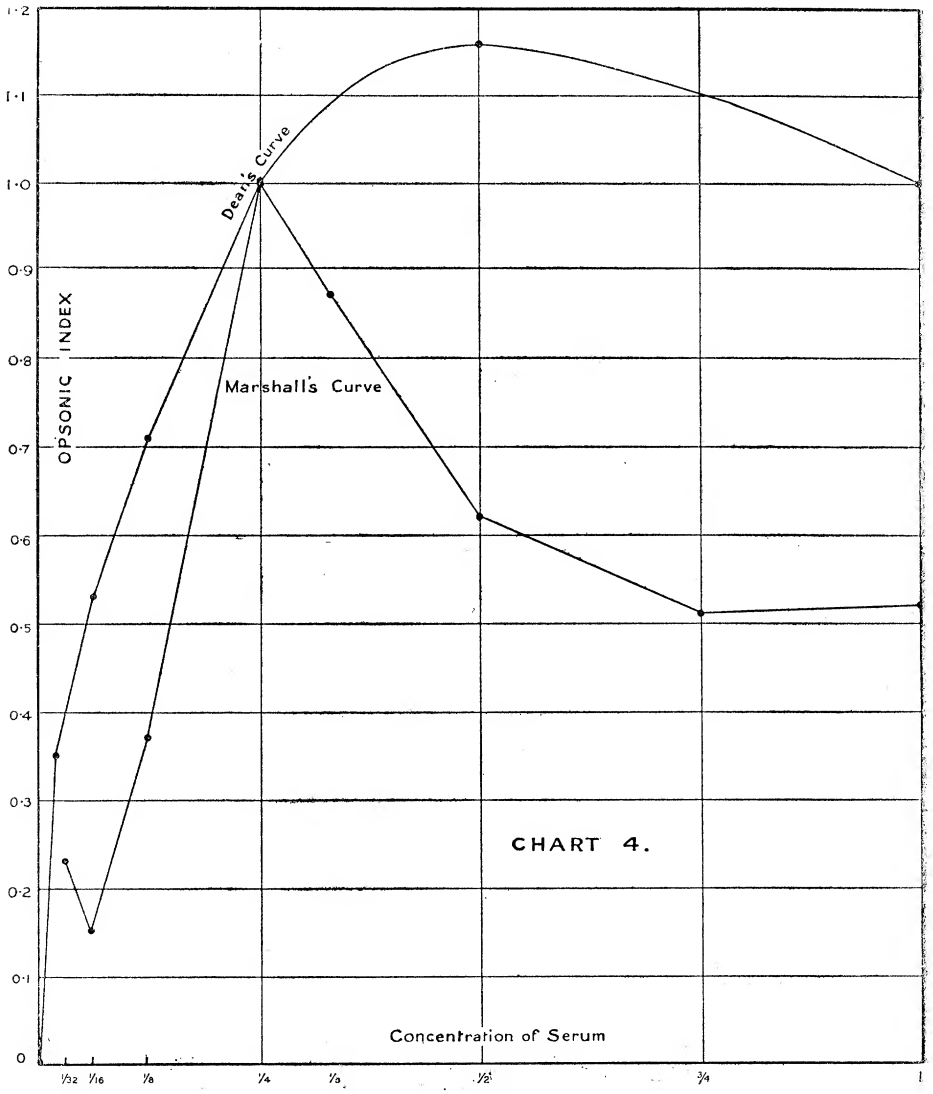
Inactive dog serum, *i.e.* y.	A. Consecutive reaction.	B. Simultaneous reaction.
c.c.		
1·0	$z = 0$	z = complete lysis.
0·5	,,	,, ,,
0·35	,,	,, ,,
0·25	,,	,, ,,
0·15	,,	= nearly complete lysis.
0·0	,,	= 0.

$x = 0·5$ c.c. guinea-pig serum. Cells = 1 c.c. of a 5-per-cent. suspension from guinea-pig.

* Ehrlich, 'Studies in Immunity,' English edition, p. 211.

VII. *Opsonins and Stimulins.*—Papers by Dean* and Marshall† contain many interesting observations on the effect of dilution in opsonin and stimulin experiments.

Chart 4 shows two curves: one from Dean, showing the effect of dilution on the opsonic index in a normal serum; one from Marshall, in the serum of



a horse, "Rich," which had been immunised against staphylococcus, the treatment covering a period of about two years.

* 'Roy. Soc. Proc.,' B, vol. 79.

† 'Journ. of Path. and Bact.,' 1908, vol. 12.

Comparison of these curves (especially that of Dean) with Chart 2 shows a marked similarity. Stated shortly, the characteristics of opsonin and stimulin reactions are as follows :—

(1) *An unheated normal* serum induces phagocytosis of organisms by leucocytes.

(2) *A heated normal* serum induces practically no phagocytosis.

(3) *An unheated immune* serum induces strong phagocytosis.

(4) *A heated immune* serum induces marked phagocytosis.

The phenomenon of phagocytosis consists of two stages: firstly, approximation of the organism to the leucocyte, probably by some amboceptoral action, possibly by chance collision; and, secondly, inclusion of the organism by the leucocyte. The leucocyte is undoubtedly a source of complement, so there may be present a zone of complement in or about it.

Let us return to the comparison between the curves on Charts 2 and 4, presuming for the moment that complement takes part in the reaction.

If complement acted proportionally to its concentration in the serum, then the experiment would appear on a plane erected on the line $y = x$.

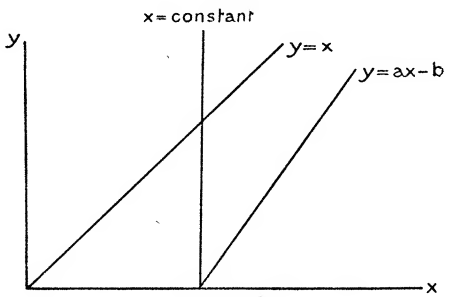


Fig 2.

Rearranging equation (8), putting $y = x$, and extracting roots, we have

$$y = \frac{cz^2 \pm cz\sqrt{z(4-3z)}}{2(z-1)}.$$

y can have only one positive value when $z<1$;

y has equal roots (*i.e.*, the summit of the curve)

when $3z = 4$, or $z = 1\dot{3}$.

Thus if lysis at the summit = 100 per cent., it cannot fall below 75 per cent. in the plane (z, $y = x$).

Marshall's curve, however, falls to 50 per cent. at least, whilst Dean's falls (within the limits of experiment) to 86 per cent.

Let us now return to the characteristics (1 to 4) above.

The fact that unheated sera act better than heated sera shows that the complement of the serum is called into play.

That a heated normal serum induces practically no phagocytosis, whereas a heated immune serum may induce a marked reaction, would suggest that the amboceptor (which is present in large quantity in the latter) acts as a stimulant on the leucocyte to secrete complement.

From this point of view Marshall's curve would lie in the plane $(z, y = ax-b)$. Dean's curve, on the other hand, which is that of a normal serum (which when heated would yield practically no phagocytosis), would lie either in the plane $(z, y = ax)$, or $(z, y = ax-b)$ where b is a very small quantity. I have calculated this curve, both in planes $(z, y = ax)$, in which case the observed value of z with full concentration lies too low, and in the plane $(z, x = b)$, in which case the observed value is too high.

Thus, to summarise, amboceptor and complement both take part in the reaction. Complement is supplied by the serum (if this source be available) or from the leucocyte, by virtue of stimulation of the cell by the amboceptor—a stimulation to secrete complement, which acts locally.

The curves of Dean and Marshall (Chart 4) are merely further exhibitions of the phenomenon of complement "diversion." (Marshall also gives curves of heated immune sera experiments which show the diversion phenomenon.)

The following points may be of use in calculating curves and finding the values of constants in the plane $(z, y = ax)$. When $y = ax$, equation (8) assumes the form

$$y = c\left(\frac{az^2 \pm z\sqrt{az(4-3az)}}{2(az-1)}\right). \qquad (17)$$

z has a maximum value when $4 = 3az$, or $z = 1{\cdot}\dot{3}/a$, and consequently $y = 2cz = \frac{8}{3}\frac{c}{a}$. z has only one positive value when $z < 1/a$. Thus $z = 1/a$ is the asymptote.

Now by the equation (10) $y = x/z$ at the "point of diversion," and consequently in the plane $(z, y = ax)$ a point of diversion occurs when $z = 1/a$. Thus if the value of z on the asymptote is known, we know its value at the "point of diversion." In whole serum experiments $y = x$, consequently $x = 1$ at the point of diversion and on the asymptote.

VIII. *The values c and z (Immunity).*—(*a*) c is a constant inversely proportional to the amount of y present in the undiluted serum.

(*b*) z is a complex factor. It is, from a chemical point of view, the amounts of x and y which have been transformed. It is in general the *effect*. It includes, degree of effect, resistance to effect, concentration of stuff

acted upon (in the special case of hæmolysis, the number of cells acted upon). In equation (1) it was used to denote the ratio x/y (see equation 10).

Thus $$z = x/y\mathrm{E}f(n)r, \qquad (18)$$

where E = effect,

$f(n)$ = a function of the number of cells, or the concentration of stuff acted upon,

r = internal resistance (*e.g.*, of the cell),

R = any external resistance, and a function of the temperature.

Since y/c and x/cz measure the total quantities of amboceptor and complement in a serum, the determination of c and z will afford absolute values for these two components; $1/c$ will be the measure of units of amboceptor, and $1/cz$ the measure of units of complement. An increase in z will correspond to a decrease or deterioration of complement, and an increase in c to a decrease in amboceptor.

In immunisation, however, amboceptor is supposed to increase, and complement to remain more or less constant. Thus in immunisation there would be a decrease in c and a corresponding increase in z. It is on this basis that indices of immunity, obtained from experiments in which both amboceptor and complement take part, must rest. The present system of bacteriolytic and opsonic indices, based on the observation of a single dilution of the serum to be investigated, is and must be liable to error. In the case of the horse "Rich" the opsonic index for the whole serum had fallen, after two years' treatment with staphylococcus, although I believe that the content in amboceptor had actually increased. I would point out that an animal such as this one, even although its opsonic (or other compound) index had decreased, would be more potent as a source of anti-staphylococcic serum than it was when its index was high. Such an animal should not be discarded without careful testing.

This fact is also of extreme importance with regard to treatment of patients by immunising. For instance, the opsonic index obtained by whole serum evaluation would fall off in the later stages of a protracted course of treatment, even although the serum had gained in amboceptoral content. I do not think that this would be a dangerous state of affairs from the patient's point of view, as possibly he may possess resistive power in a potential state, but the fact is worthy of note and of research.

With regard to the value $f(n)$. Throughout this paper, and in my experiments, I have taken n to be sufficiently great to absorb all the amboceptor used. But other conditions may prevail. For instance, in opsonic experiments there is a limit in the size of the cell, which would

present itself, if only a very small number of them was present. In agglutination experiments, if the organisms were few in number, a portion of y would remain unabsorbed.

IX. *Toxins and Antitoxins.*—Ehrlich has postulated that the toxin molecule has two receptors, a toxophorous, which is comparatively thermolabile, and a haptophorous, which is thermostable. Arrhenius has stated that these receptors may be considered to belong to two different molecules. I shall go a step further, and by imitating the chief characters of toxins, and their neutralisation by antitoxins, show that these two factors are probably amboceptor and complement, and that the antitoxin is an anti-amboceptor:—

(*a*) A primary characteristic of toxins is that different samples may agree in their minimal lethal dose (M.L.D.), whilst they disagree in the amount of antitoxin necessary for their neutralisation.

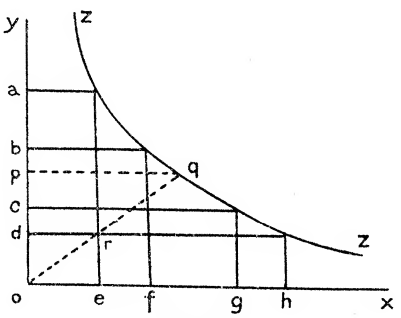


Fig 3.

Fig. 3 is a form of chart obtained as on Chart 1, z = effect (in Chart 1, z signified complete lysis, *i.e.* death of the cell. It may now be considered as a "lethal" line).

Toxins consisting of—

$$ay+ex, \qquad cy+gx,$$
$$by+fx, \qquad dy+hx,$$

all have the same lethal effect, but the quantity of an anti-amboceptor requisite for neutralisation would be a, b, c, d, equivalents respectively.

(*b*) A second characteristic is that a toxin which has deteriorated in lethal effect still requires the same amount of antitoxin to neutralise it. This is obviously a corollary of (*a*).

For instance, let a toxin $dy+hx$ deteriorate to $dy+ex$; d equivalents of anti-amboceptor will be required for neutralisation of both the fresh and the deteriorated states, but the lethal effect will have been much diminished. As amboceptor has been considered to remain constant during deterioration,

the lethal dose may be measured in terms of amboceptor. In the fresh state the dose is measured by *od*, and in the deteriorated by *op* (fig. 3).

(*c*) A third characteristic is in the curious curve which toxicity (as ordinate) assumes, as increasing quantities of antitoxin (as abscissa) are added. The curve is an $\int$ written backwards. At first it is almost flat (ascribed by Ehrlich to prototoxoid), then it descends more sharply, and finally it becomes again flatter. Arrhenius has shown that the flat finish can be explained on the principle of mass action equilibria between toxin and antitoxin, but the flat commencement cannot thus be explained.

Toxicity is inversely proportional to the minimal lethal dose, and is to be distinguished from *z*, which means degree of effect.

Minimal lethal dose can be shown in fig. (3) as follows: Let 1 c.c. of a toxin contain $dy+ex$. We can measure this by *or*. Thus $or = 1$ c.c. Now, to cause death, *or* must be produced to *oq*, the lethal line, that is to say, the minimal lethal dose is oq/or c.c., and the toxicity $= 1/\text{M.L.D.} = or/oq$.

Now suppose we have a toxin containing $ay+ex$ in 1 c.c., and that, after acting on it with anti-amboceptor, *ay* is reduced to *dy*.

The lethal dose of $ay+ex = 1$ c.c. (*vide* fig. 3),

but the lethal dose of $dy+ex = (oq/or)$ c.c.

The toxicity is or/oq.

If we assume, as a simple case, that *ay* is neutralised by *a* units of antitoxin, then the curve of toxicity is flat at first, and becomes more and more steep as more antitoxin is added. But if we assume with Arrhenius that complete neutralisation never occurs, then this curve is drawn out in its final part, and never reaches the abscissa.

Thus the prototoxoid phenomenon will appear in certain cases, and I would add that it is possible that the curve might even commence with a rise, *i.e.* a toxin might be found which became apparently more toxic on addition of a small quantity of antitoxin. I am not aware that this has been observed.

These three primary characteristics of toxin and antitoxin have thus been shown to be fully described by the equilibrium relation of equation (1) taken in conjunction with Arrhenius's views; the haptophorous group being represented by amboceptor, the toxophorous group by complement, and antitoxin by anti-amboceptor.

Summary.

1. Amboceptor and complement are opposed in their action on the cell, with the proviso that the former acts as a catalyst to the latter.

2. Complement action is lytic; amboceptoral action is primarily polymerising, or, as the case may be, agglutinative; and, secondarily, catalytic to complement.

3. The relation of these substances is expressed by the law of mass action, in the form

$$\frac{dz}{dt} = \frac{y}{c}\left(\frac{x}{cz} - z\right) - \left(\frac{y}{c} - z\right)^2.$$

4. When the substance acted upon is in sufficient quantity, this expression describes all serum reactions, viz., hæmolysis, bacteriolysis, opsonin and stimulin reactions, agglutination, precipitation and toxin action.

5. Toxins are compound and consist of amboceptor and complement.

[*Note.*—The idea that amboceptor and complement were opposed in their actions has been held by Captain W. F. Harvey and myself for many years. We joined in many experiments to prove our idea. It was only recently that I determined to investigate diversion phenomena from a mathematical point of view. I did so, and found, at the end of my work, that I had again to have recourse to this same view of opposed action, a result which I had not foreseen. Captain Harvey and I were in the habit of calling amboceptors *polymerins* and complement *lysin*, and I know of no better names to describe their properties. Amboceptoral action is always, when acting alone, a polymerising one, whereas agglutination may, or may not, occur in the course of polymerisation. Agglutination is a molar, whereas polymerisation is a molecular phenomenon. Indeed, as has been shown by Muir, reduction of polymerisation by complement may cause agglutination.]

I have also to express my thanks to Major Cornwall and Dr. Kesava Pai, the Director and Assistant Surgeon of this laboratory—the Pasteur Institute of Southern India.